Design, construction and validation of a Control system of the Physiological properties in a Cardiac Bioreactor

Bachelor Thesis

Degree in Biomedical Engineering
Bioengineering and Aerospace Engineering department

Author: Ander Vallinas Prieto

Tutor: Andreu Martínez Climent

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The friends who have accepted to read this work through and give me feedback even when they did not know nor understand anything about the topics presented have my gratitude too.

Finally I would like to acknowledge my parents, who have never doubted that I would make it, for being my best inspiration for life.

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1. Abstract

During this Bachelor Thesis a system to measure, record and control physiological properties in a cardiac bioreactor has been designed, developed and validated. The present study has been developed in the Laboratory for Bioartificial Organs of the Hospital Gregorio Marañon of Madrid.

The cardiac bioreactor for which this system has been built keeps an isolated pig heart alive and beating during several hours by using a modified Langendorff system. The main goal of this bioreactor is to evaluate the mechanisms of mortal cardiac arrhythmias under a control situation and without producing any kind of pain in animals. Specifically, porcine hearts are maintained alive by forcing coronary arteries perfusion with a physiological solution that mimics blood. The main goal of the present study was to develop a system of sensors to have physiological properties of the perfused media under control to foster the proper functioning of the cardiac processes taking place in the bioreactor.

During the development of the present bachelor thesis, a system that measures oxygenation, pH, temperature and level of the circulating medium in the main vessel of the bioreactor has been developed. In addition to record and store the data, the system trigger specific alarms if any of these properties is out of a user defined range. The developed prototype makes use of self-made MATLAB software, a camera and an Arduino board connected to three sensors, an LCD screen and a buzzer.

It has proven to work for long periods of time without problems, measuring properly all these properties and triggering the alarm when they were out of user defined range.

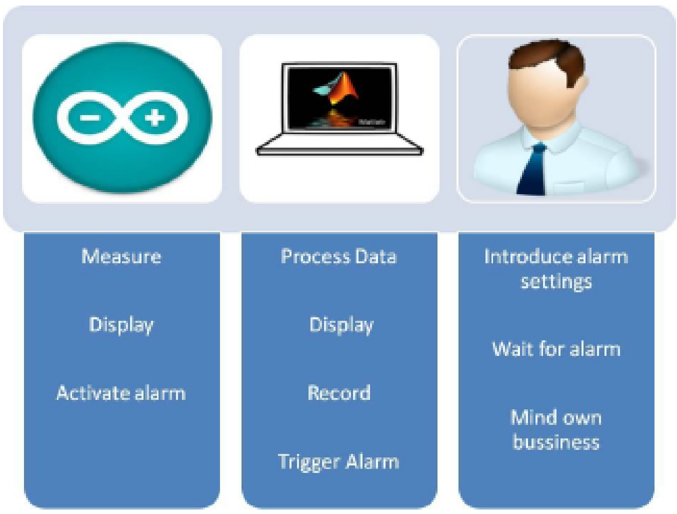

Figure 1: Roles of the parts of the system

2. Introduction and Background

2.1. Bioreactors

According to the International Union of Pure and Applied Chemistry (IUPAC), a bioreactor is "an apparatus used to carry out any kind of bioprocess" [1]. This is a rather broad concept that engulfs a lot of processes from biotechnological applications like those designed for carrying out enzymatic reactions, which are the ones commonly associated with the term "Bioreactor", to those that are used for cell culturing or ex-vivo tissue and organ survival systems. In order to design an efficient bioreactor several parameters must be taken into account, as optimum conditions of temperature and pH and the behavior of cellular metabolism, because nutrients will display a spatial profile through the reactor and it will be affected [2].

There are several types of bioreactors, including artificial and natural ones as calf stomachs [2]. Artificial or commercial ones have three main categories: non-stirred non-aerated systems, non-stirred aerated systems and stirred aerated systems [3]. First type are fermenters, second are typical culture flasks, that by themselves do not stir the contained medium and third are the ones in which all the spatial concentration of nutrients is tried to be homogeneous.

The third type of bioreactors aforementioned has several variants depending in the way the air and medium is distributed. There are simple stirrers, which provide a circulating pattern, also those that use pressurized air to produce the circulation or those that use pumps to control the flow [3].

This Bachelor Thesis is centered on a Langendorff system. This system is a pump driven stirred aerated bioreactor. It is a system that keeps an isolated heart alive and is used as animal model to study cardiovascular disease [4]. It was first described in nineteenth century and allows for the study of phenomena in controlled conditions accessing directly to the regions of interest. Hearts of different species can be used from rats to big animals as pigs or even humans. To keep the heart alive, a tube is introduced through the aorta and a medium is circulated, and advancing along the coronary arteries it irrigates the myocardium and is drained by the venous system. The circulated medium contains known adequate proportions of electrolytes and glucose, following the Tyrode formula. The temperature, pH and the flow of the media are kept constant and it is oxygenated. The system is composed of several parts shown in the following picture:

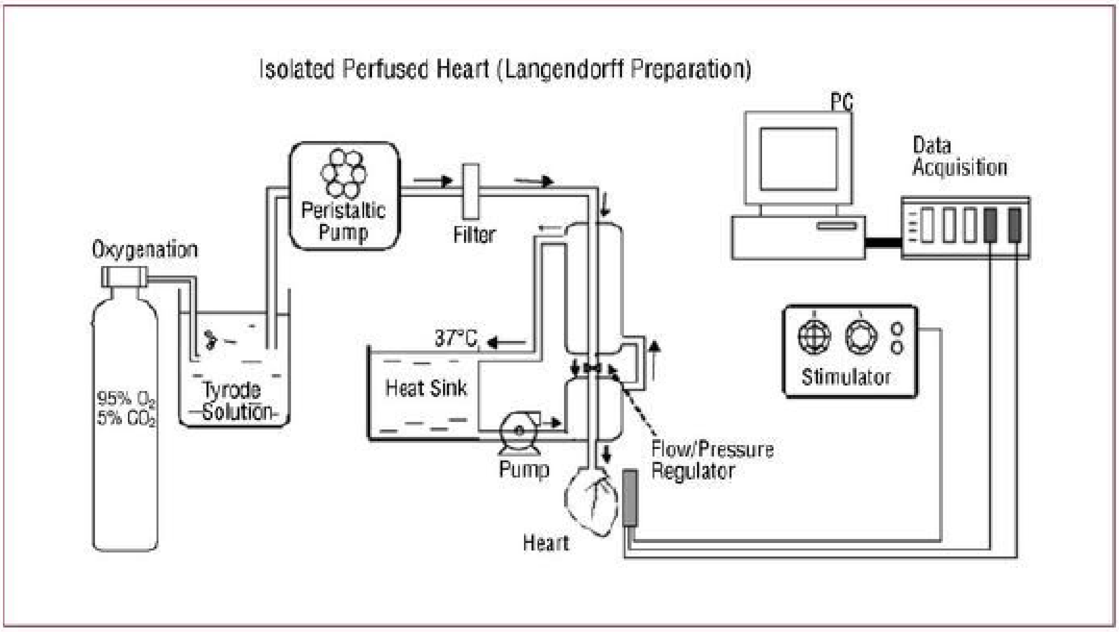

Figure 2:Schematic representation of the components of a Langendorff System. Extracted from: Modelos animales de enfermedad cardiovascular [3]

In Figure 2 it can be seen a setup of a Langendorff system, where the Tyrode medium on a main vessel is oxygenated using an O_2 tank, pumped through a filter and circulated across the heart and back. This circulation loop is complemented with another a loop that covers the main one, where hot water is circulated in order to heat up the medium. Additionally a stimulator and data acquisition system can be seen to be in direct contact with the heart using electrodes. In the specific system in which this bachelor thesis is focused, the heating system uses an independent circuit that goes through the oxygenation filter.

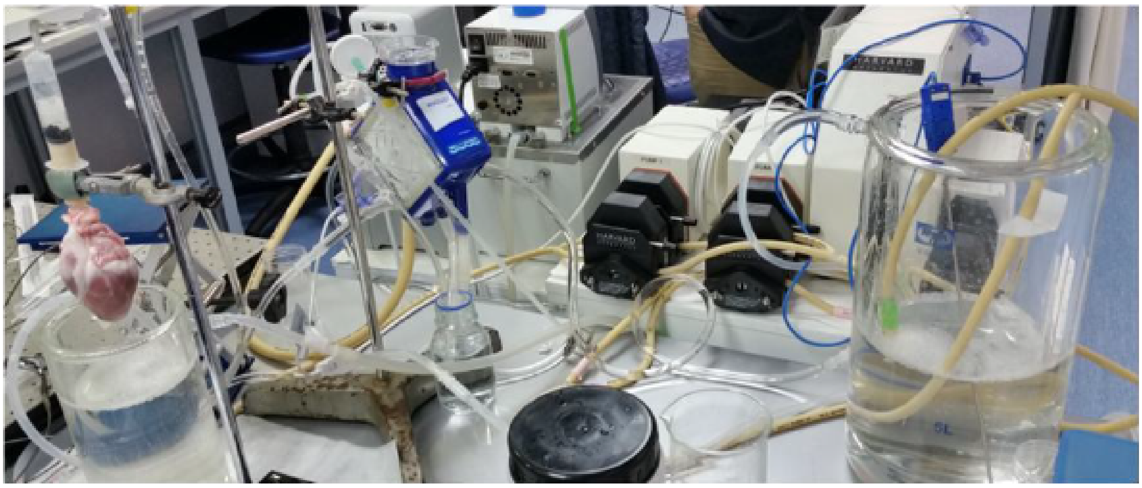

Figure 3: Picture of the mounted system in the Laboratory of Bioartificial Organs and Matrices of the Hospital Gregorio Marañón of Madrid

The main purpose of the system that is mounted in LOBA (Laboratorio de Organos y Matrices Bioartificiales) part of the Gregorio Marañon Health Research Institute (Figure 3), is to do research and gather information and more insight on arrhythmia development and further complications. The Langendorff system gives them the opportunity to test and evaluate life threatening situations as extreme doses of drugs or mortal arrhythmias without inflicting damage to the animal in a controlled situation, getting rid of ethical issues and uncontrolled factors found in animal experimentation. It also permits the usage of analysis techniques that are not feasible in vivo as the optical mapping later explained.

2.2. Cardiovascular System

The cardiovascular system has the function of distributing oxygen and nutrients to all the cells in the body and take out all the waste products of cell metabolism as CO_2. It is composed by the heart, which is the driving pump for the whole flow through the body, and by the blood vessels or vasculature. The heart works as a double pump, driving deoxygenated blood coming from the whole body to the lungs, and oxygenated blood that came from the lungs to the rest of the whole body to deliver oxygen and nutrients. The cardiac muscle cells on the heart layer called myocardium, or cardiomiocytes for short, are the ones responsible for the pumping. They are striated, but differ from skeletal muscle cells in the fact that they are auto rhythmic, meaning that they contract in a paced involuntary way. The coordinated contraction of the heart cells results in the heart beating, and that is how it fulfills its pumping task [5].

To produce a correct coordinated contraction the heart is electrically activated from the Sinoatrial node, that fires its action potential and the pulse is conducted by the myocardial cell fibers and laterally spread through connections called intercalated disks, having a determined pattern of conduction in healthy hearts [6]. After the action potential in a cardiomyocyte, contraction happens. After an action potential, a refractory period takes place, during which the cell cannot be excited again (Figure 4). This prevents the appearance of different conduction patterns that may produce strange ways of beating. Changes in the conductivity of the tissue or the appearance of obstacles as scarred tissue due to infarctions can lead to wrong patterns of conduction, leaving the heart out of normal rhythm or in arrhythmic state. This term engulfs fast beating (tachycardia), slow beating (bradycardia) or irregular beating like atrial or ventricular fibrillation [5, 6, 7] and may be also developed by abnormalities in impulse formation apart from problems on conduction, and from a combination of both too. Whether they present reentries (rotational or backward propagation of the pulse due to slow conduction overcoming refractory period of the previous and adjacent cells) or not, the criteria for diagnosing arrhythmias are not yet clearly defined. Spatial and temporal mapping of the pulse propagation can give further insight and determine these criteria [7].

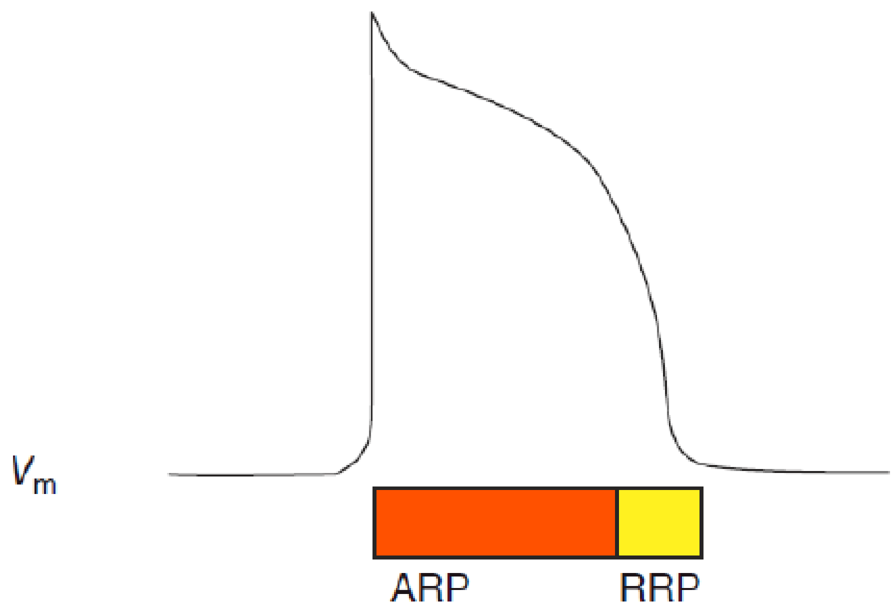

Figure 4: Depiction of an action potential with the Absolute and Relative Refractory Periods (ARP & RRP). Extracted from Basic Cardiac Electrophysiology for the Clinician [7]

This type of mapping can now be achieved in animal models like the Langendorff system with a high resolution by optical mapping [4].

2.3. Introduction to Optical Mapping

The used optical mapping method is pretty similar to the one used by Nanthakumar et al. in “Optical mapping of Langendorff-perfused human hearts: establishing a model for the study of ventricular fibrillation in humans” [8]. In the case of the current experiment the hearts are coming from pigs rather than from human patients, but the setup is pretty similar, using a Langendorff system to keep the heart alive, as detailed before. The potentiometric dye, for example di-4-ANEPPS, was perfused in the heart. This dye allocates in the cell membrane and its spectral properties change with different transmembrane potentials, emitting variable intensity fluorescence when excited in function of the transmembreane potential.

This dye is excited at a certain wavelength (i.e. a band centered at 531nm) and emits fluorescence when the action potential is triggered in each cell. In order to analyze the behavior and the conduction of the action potential wavefronts a couple of high potency LEDs with the appropriated wavelength are pointed to the heart achieving an homogeneous illumination in the exposed face and a high resolution camera is used to record the fluorescence. In Figure 6 can be seen the disposition of the camera and LEDs with respect to the heart. The camera uses a filter that blocks the wavelength emitted by the lasers because the intensity of the fluorescence signal is very low in comparison to the one of the LED. The experiment is carried in dark conditions to reduce sources of noise. This way the camera is able to record a high resolution area with high temporal resolution (e.g. 1000 frames per second), giving the possibility to select single pixels and obtain action potentials over time in single spatial points (Figure 5,Figure 7), or to identify rotors and calculate conduction velocities. The heart can be stimulated using electrodes and try to direct the electrical activity as shown in Figure 2.

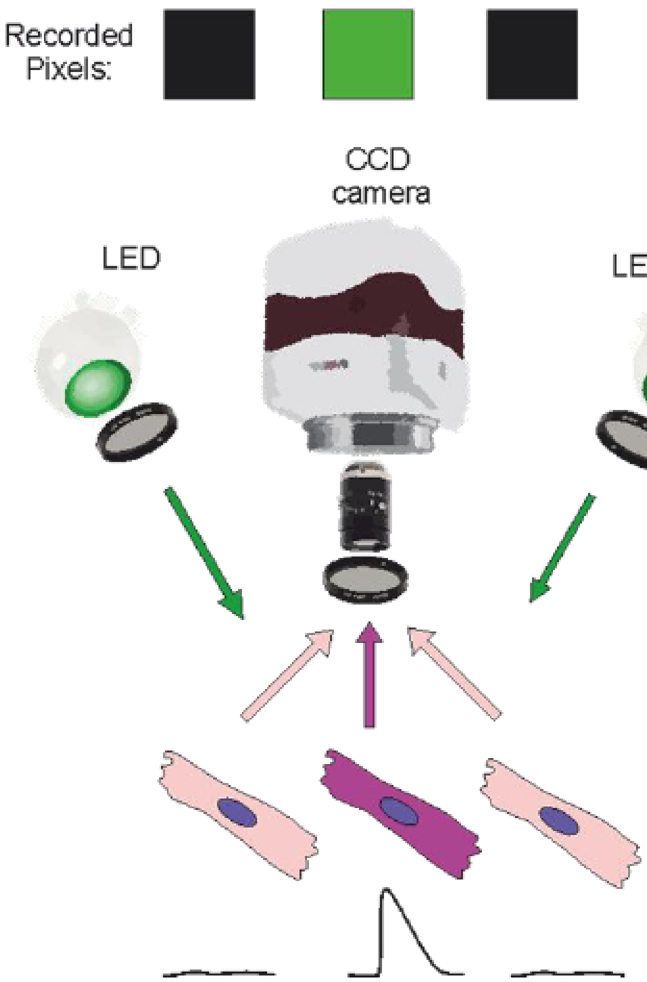

Figure 5: Optical mapping system schematics, the cells are excited by the LEDs and the camera records in each pixel if action potential is triggered

All these recordings can give insight in the developer mechanisms of arrhythmia and further complications because they give the opportunity to make in depth analysis of the data on a selected region of interest and its evolution on time. This type of setup gives the possibility to evaluate the influence of certain drugs and to study animals with pathology models as chronic infarctions.

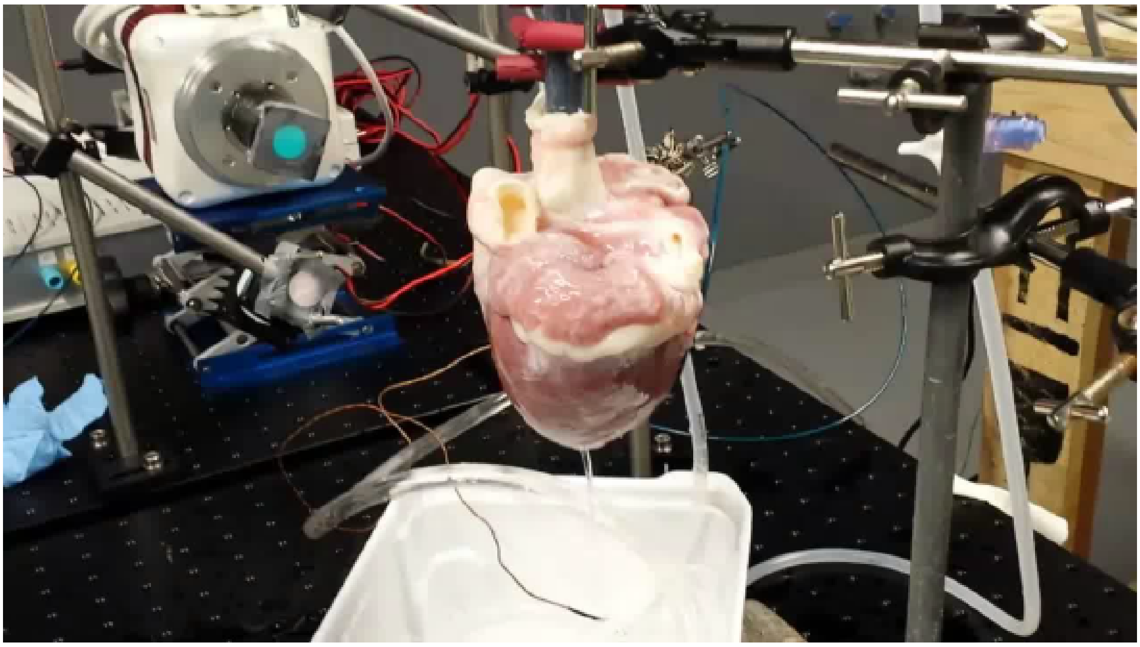

Figure 6: Camera and LED placing for optical mapping

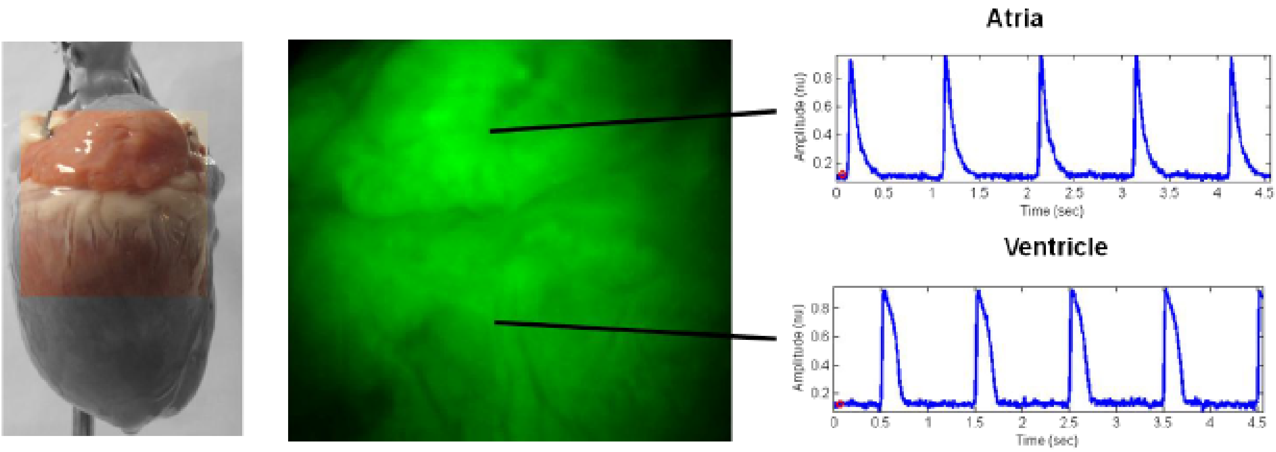

Figure 7: Results of optical mapping in a selected ROI (Region of Interest) and action potential signal over time in selected pixels

2.4. Motivation

This project was in charge of designing a system to measure parameters governing the good functioning of the Langendorff system in real time. These parameters are pH, temperature and proper oxygenation of the medium, along with the level of liquid in the main vessel.

Variations in temperature and pH affect protein folding and if they go out of homeostatic bounds may produce denaturation [9]. This can escalate to cell death and therefore organ malfunction and tissue necrosis.

An inefficient oxygenation forces cells to follow anaerobic metabolic pathways for energy obtaining and may be insufficient for cellular processes to be kept up functioning.

Level measurement is a good way to infer if there is any type of leakage in the connectors and tubing carrying the circulating medium. This, apart from producing a loss of medium, could mean entrance of air in the system that may occlude the coronary artery and develop an acute infarction.

Having these parameters under control to avoid malfunction and keep record of non-understood changes on experiments was the main motivation of this bachelor thesis. The heart is affected by these parameters of the medium and the medium is affected also by the heart as it is circulated, so keeping track of them is a sensible and maybe enlightening idea. It is a sensible idea because heart cannot maintain homeostasis by itself. It is not connected to the endocrine and central nervous systems, which are the major control systems to control homeostasis in vivo [10].

There exist devices to measure all these parameters individually but the aim was that this system measured all the parameters together and over time, not just once, displaying them and keeping a record.

3. Objectives

The main aim of this bachelor thesis was to design, develop and validate a system to measure, record and control pH, temperature, level of medium and oxygenation in the cardiac bioreactor of LOBA. To do so the following specific objectives have been selected:

- Design and develop a unit of control of the pH of the medium.
- Design and develop a unit of control of the temperature of the medium.
- Design and develop a unit of control of the level of medium in the main vessel.
- Design and develop a unit of control of the appropriate oxygenation degree.
- Assemble a complete system capable of registering and storing the controlled physiological parameters.
- Design a graphic user interface to support the user from where the system can be controlled and the alarms programmed.

4. Materials and Methods

In the following section a brief description of the components of the system and their function is going to be done, along with the tools that were used in the system assembly and the methodology used to test its correct behavior. First the way each parameter was measured is explained. Second the assembly of the system and the graphic user interface development is described. Next the physical assembly of the circuit and the used software is exposed and finally the carried out experiments are detailed.

In Figure 8, a scheme of the whole system is depicted. All sensors are controlled from the same MATLAB user interface. The communication with three of them (pH, Temperature and Level measuring) is performed by using an Arduino Board, whereas the oxygenation check is made by using a digital USB connected camera directly connected to MATLAB. For further description of the system and how to use it manual, go to: Annex I: Project User Manual.

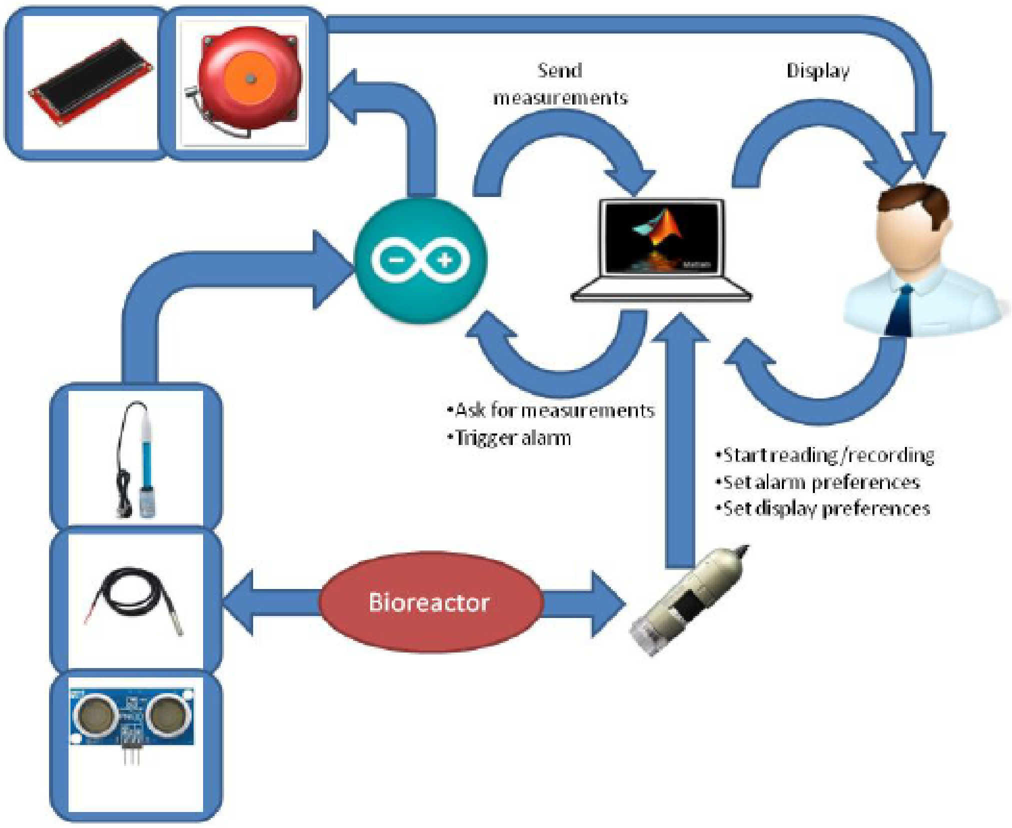

Figure 8: Schematics of the system

4.1. Measurement of pH:

The pH measurement was done with the sensor DFRobot SEN 0161. This sensor is a pH probe, with a glass measurement electrode and a silver/silver chloride reference electrode within. In order to measure, the probe has to be in contact with the solution. This sensor measured a voltage and transferred it to Arduino. Arduino divided the range of 5V into 1024 steps, and got a value from 0 to 1023. In order to obtain a proper measurement Arduino read 10 measurements from the analog port where the sensor was connected, sorted them in increasing order and averaged the 6 central values (discarded the first two and last two values). Then it transformed the averaged number to voltage again, and finally it converted voltage to pH. This sensor in particular has the following transfer function:

$$pH = 3.5 * V_{measured} + pH_{offset}$$

$$V_{measured} = measurement * \frac{5[V]}{1024}$$

4.2. Measurement of Temperature

For the temperature measurement the digital sensor DS18B20 was used. This sensor was introduced directly on the solution and retrieved a value when Arduino asked for it. The obtained value was directly a temperature, because the own commands of the specific library for this family of sensors was used.

In order to measure temperature this device makes use of two oscillators, one with low temperature coefficient and the other with a high one. It counts the number of oscillation cycles completed by the low temperature coefficient oscillator during a gate period determined by the high temperature coefficient oscillator. As the measurement is digital, slope-overload distortion may occur and for correcting this error a slope accumulator circuit is used, retrieving a high resolution (<0.05ºC) in the measurements. Then it converts the measurement to 2-byte length binary data and retrieves it to the microcontroller on request.

4.3. Measurement of Level of Media in the Reservoir

In order to measure the liquid level of the vessel the ultrasonic sensor PING SEN-0012 was used. The sensor was placed facing its transducers to the surface of the liquid solution. Every time it was needed to measure the level Arduino sent an ultrasonic pulse using the transducers, this pulse was reflected backwards when collided with the liquid surface and was sensed by the transducer. A schematic depiction of the physical principle used by this sensor can be seen in Figure 9. Arduino measured the time that the pulse took to come back since it was sent. Knowing the sound speed and that the travelled distance by the pulse is twice the distance from the transducer to the surface (forwards and backwards) using the measured time, the distance to the surface can be calculated and subtracting it to the total vessel height the level can be calculated:

$$Level\ [cm] = distance_{empty\ vessel}[cm] - \frac{pulse\ duration\ [\mu s]}{2} * \frac{1}{29}\ [^{cm}/_{\mu s}]$$

Using SolidWorks software a piece was designed to support the sensor facing the liquid surface, and was printed using a MakerBot Replicator 2 3D printer with PLA (Poly Lactic Acid) as material.

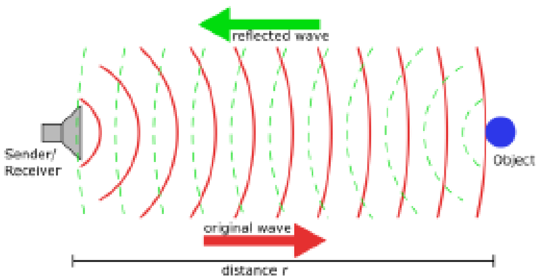

Figure 9: Physical principle of ultrasound distance measurement, same velocity for the original and the reflected waves

4.4. Oxygenation Check: Method and Calibration

With the objective of keeping the oxygen flux up through the whole experiment a qualitative check method was developed. With a camera, pointing to a small recipient of media where the oxygen was discarded while it was flowing, the presence of ascending bubbles was checked. To do so, ten images were taken in a short period of time and their correlation was calculated (each with the next one, giving 9 values of correlation). A high correlation value means that the images are pretty similar and bubbles flowing would reduce this similarity, yielding a lower correlation value. A threshold was sought for which the correlation implied no bubbles flowing above this value.

However the system was thought to be used in low light intensity situations. In completely dark situations the camera was not able to register images in which the bubbles could be distinguished, but it could do so with the intensity provided by the LEDs. Another threshold for light intensity was sought above which the measurements could be recognized as valid (bubbles could be distinguished above that intensity value).

To obtain the intensity threshold the oxygenation valve was left open and a video was taken. During the video the LEDs were turned on and off several times and the correlation of all the frames with the following ones was calculated. For those sets of frames with a maintained low correlation the average intensity of each image was calculated and the minimum was taken as a threshold. In order to obtain the correlation threshold the LEDs were lit and a video was taken. During the recording the oxygenation valve was opened and closed several times. The correlation of each frame with the following one was calculated and a minimum correlation value for non-bubbly set of images was taken as threshold.

If the light intensity on the image was not high enough these correlation were discarded, and if the correlation was high enough it meant that there were no bubbles changing of place from one picture to the next one.

Finally to ensure the stability of the measurement, it was checked that at least three out of four consecutive correlation values were above the threshold to determine whether the oxygenation valve was closed or open and therefore avoid false positive and false negative outcomes due to variations in the valve opening and closing and the possibility of having bubbles in similar places from one frame to another. A flowchart of the described algorithm and a scheme of the camera placing is shown in Figure 10.

The used camera was a DINO Lite AD4113TL portable microscope.

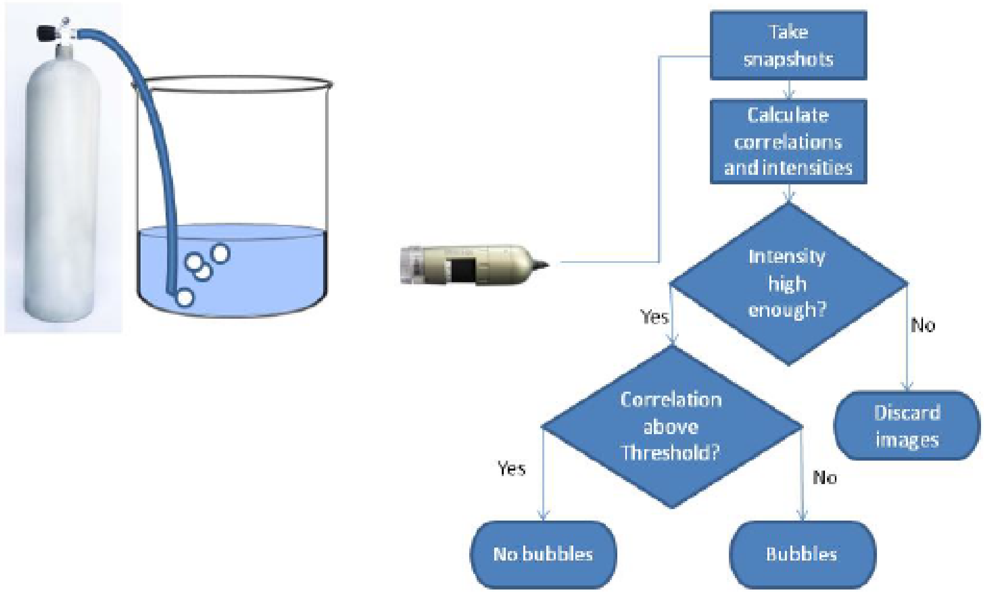

Figure 10: Schematics of the camera placing and flowchart of the checking process

4.5. System Control and Measurement Processing

All the system was controlled by a MATLAB program with a customized user interface. This program was developed with MATLAB programming language and the GUIDE tool, used to generate Graphic User Interfaces. It was in charge of requesting periodical measurements of pH, temperature and level to Arduino when needed, displaying the measurements (graphs with time evolution and last measured value in text) and checking that they were within the range defined by the user and if not ask Arduino to trigger an alarm in the following measurement request. It also checked the oxygenation directly and triggered the alarm in the same way if a camera was available and the user wanted so. It made a record of the measurements when requested to. The display was editable in the sense that the user was able to select the time scale of the displayed graphs and also define the limits of the measurement axis.

To get details concerning all the possibilities the program allows a Project User Manual can be found in Annex I. To be short in explaining the communication protocol, an Arduino sketch was programmed to control the Arduino UNO board. Arduino was connected to the computer using a USB port and the communication with MATLAB was managed through serial port method. Arduino was in charge of retrieving the measurements of pH, temperature and level, display the last measurement in an LCD, and send the measurements back to MATLAB and triggering an alarm if MATLAB, after processing the measurements, found that some conditions predefined by the user were met.

4.6. Design of Prototypes

The prototypes followed several development stages. First the way that the circuit needed to be connected was tried on a protoboard, to check that the devised connections were correct. Next step was assembling the whole system on a breadboard, where the connection trails replacing wires were soldered with tin according to the circuit needs. Finally a PCB was designed using Fritzing software and is ready for manufacturing as a next step of development.

4.7. Evaluation of Sensors Performance

In order to prove that the sensors were working properly, their measurements were contrasted with respective gold standards.

In the case of the pH and temperature sensors the measurements were compared with the ones of "Eutech Instruments" pH700 model which has a pH-meter and a temperature probe. On a solution where the pH was changed adding droplets of diluted HCl and NaOH 8N, both measurements were noted down for comparison.

The temperature sensors measured the temperature of some water that was slowly heated up using "Fisher Scientific" Heating Magnetic Stirrer FB15001 model, and also were noted down.

The level sensor was tried on a vessel in which liquid was added and withdrawn manually and compared with the measurements of a regular ruler.

In the case of the camera algorithm for oxygenation detection the gold standard was the user's sight, which could determine whether there was carbogen gas flow or not.

4.8. Trial of the whole System

The system was assembled and placed in position for measurement. The program was run for long durations and all the editable settings and functionalities were overlapped in order to find out remaining bugs through the code and try to successfully operate for several hours during isolated heart experiments.

5. Project costs and working time estimations

In the following section the cost of hardware needed for the project along with working time estimation is presented. Also the enabling technologies available for the student and needed to develop this project are mentioned.

5.1. Component and tool costs

In order to determine costs, the components were sought on the internet and the following table shows approximated costs for each component, as their price can vary from one store to another. The laptop amortization cost was calculated assuming that a computer costs 1000€, has a useful life of five years and it has been used during approximately half a year. A similar approach has been used for the fees of software calculation, retrieving an approximate share proportional to the time they have been used.

Component	Cost (approximated, cited 7 June 2015)
Arduino UNO	22€
DFRobot SEN 0161	≈26.8€
DS18B20	3.49€
PING SEN-0012	30€
DINO LITE AD4113TL	≈437.8€
Breadboard	≈2€
Buzzer	≈3€
3xResistors	1€
PLA for piece printing	1.5€
Fee for temporal use of MATLAB License	60€
Fee for temporal use of SolidWorks Standard	50€
Arduino Software	Free
Laptop amortization cost	100€
Total	737.59€

5.2. Person Hours

These are estimations on costs of having a biomedical engineer involved in this project working for the time it supposed. It accounts estimations on wage, electricity costs, water costs, insurances, rentals, administrative staff and supervision.

Job	Hours	Cost per hour	Total Cost
Biomedical Engineer	360	25€	9000€

6. Results

In this section the outcome of the experiments and processes detailed in Materials and methods is explained. First is explained the performance of each individual sensor, second the threshold determination for the oxygenation check algorithm. Next the results for the system assembly are depicted and finally the behavior of the whole system in normal conditions and forced situations is explained.

6.1. Sensor performance

PH Sensor

The sensor used in this project can be seen in Figure 11:

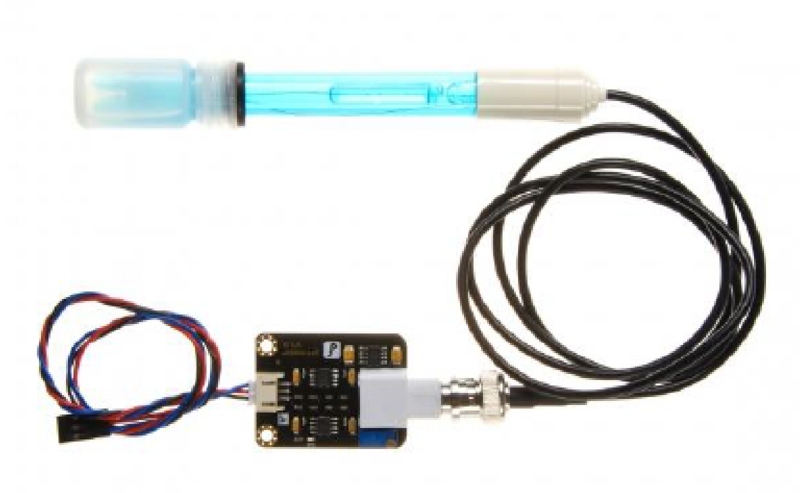

Figure 11: DFRobot SEN0161 pH Sensor

The linear fit made with Microsoft Excel gives a R^2 coefficient of 0.95, which means that it approximates well to a linear behavior despite the non-linear curve depicted in Figure 12, and the slope is close to 1 but, it presents a zero drift of -1.19. This retrieves lower measurements than needed and taking into account that the scale of pH goes from 0 to 14 this is a very high factor.

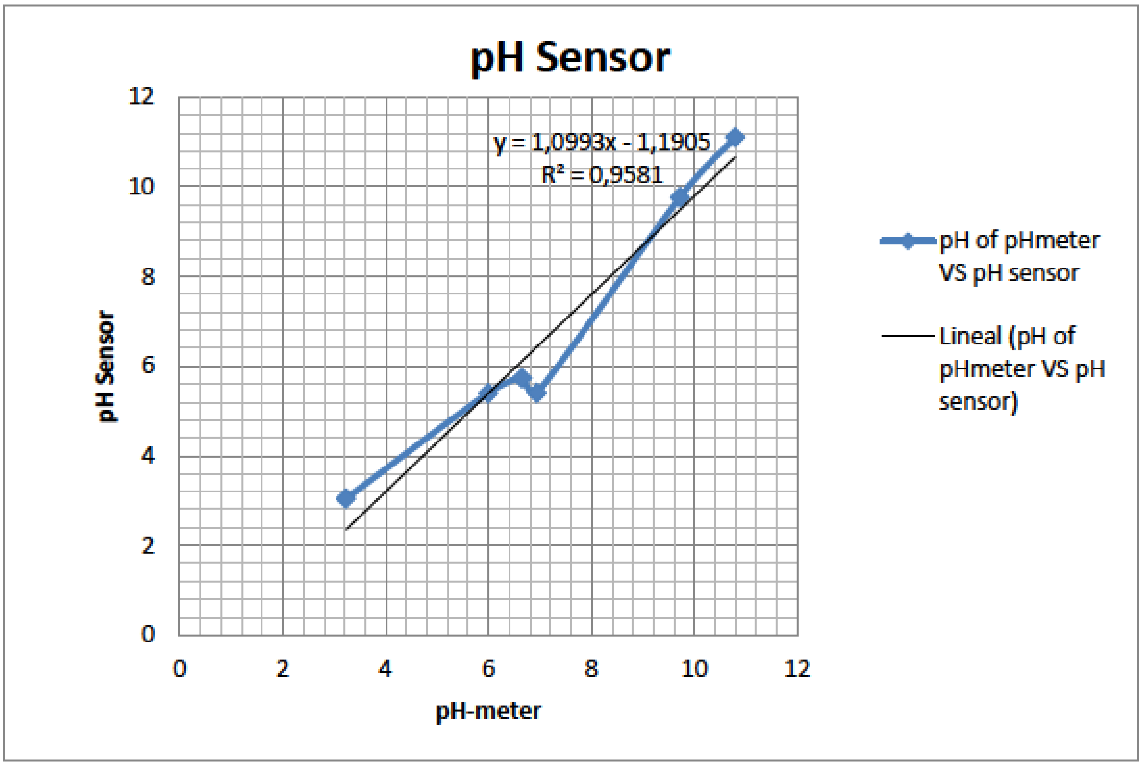

Figure 12: Measurements of pHmeter (gold standard) versus the pHSensor used in this project in the same vessel, while pH was being changed

Temperature Sensor

The used temperature sensor is depicted in Figure 13:

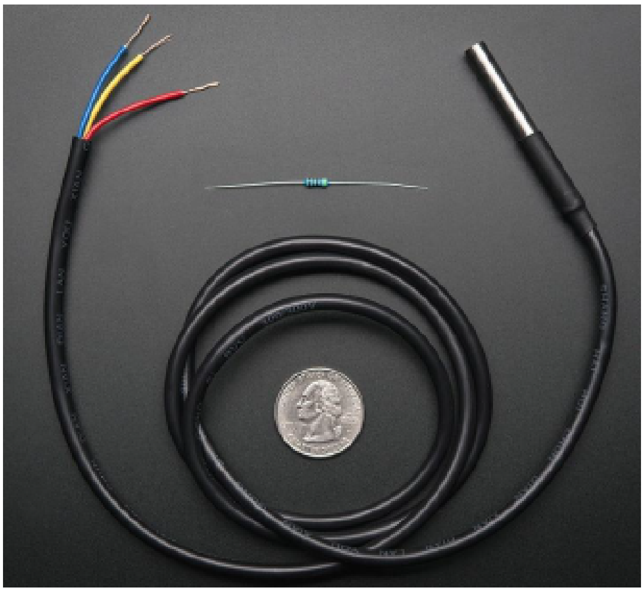

Figure 13: DS18B20 waterproof temperature Sensor

In the case of the temperature sensor, the response to a continuously increasing temperature on solution in comparison to a lab thermometer showed a faster pattern, always measuring one or two degrees less than the thermometer, which is not very accurate though. However it showed an almost linear pattern as showed by the tendency line and the R^2 value from graph depicted in Figure 14.

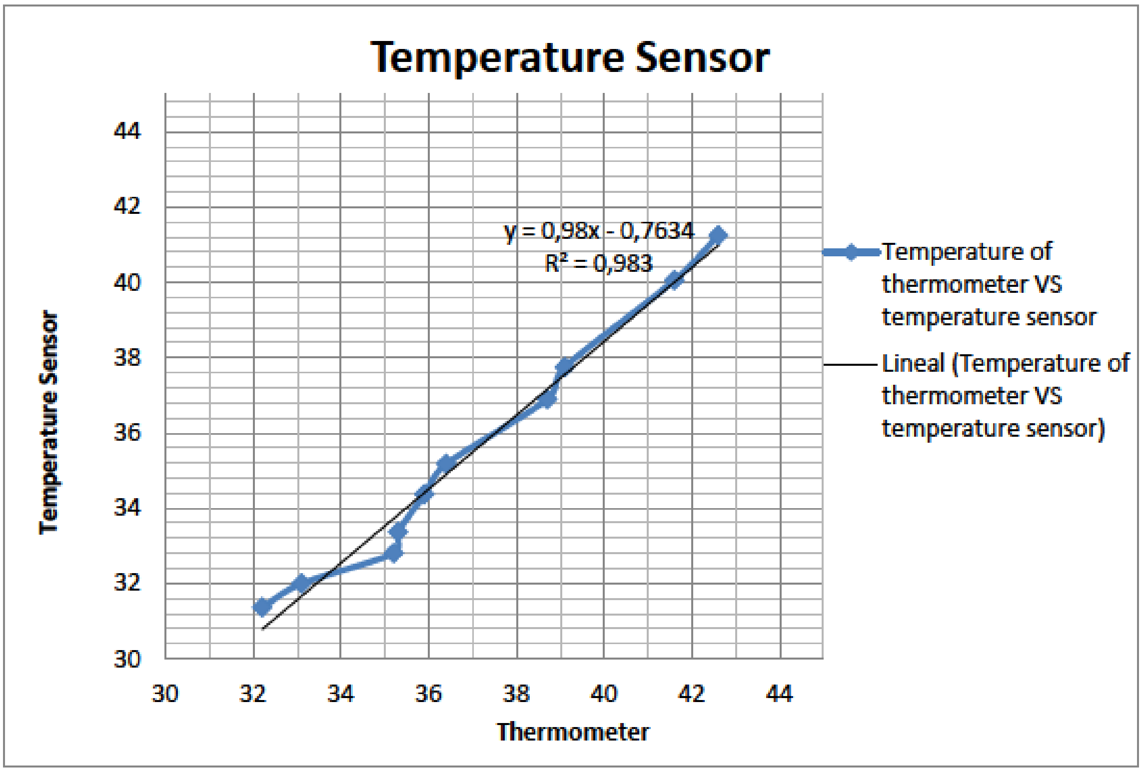

Figure 14: Measurements of thermometer (gold standard) versus temperature sensor used in this project the same vessel while fluid was heated up

Level Sensor

In Figure 15 can be seen the used sensor for the level measurements:

Figure 15: PING SEN-0012 US proximity sensor

The designed piece for the US sensor was correctly printed (Figure 16) and fit perfectly with the sensor. The support task for which it was devised was fulfilled, as it can be visualized in Figure 17.

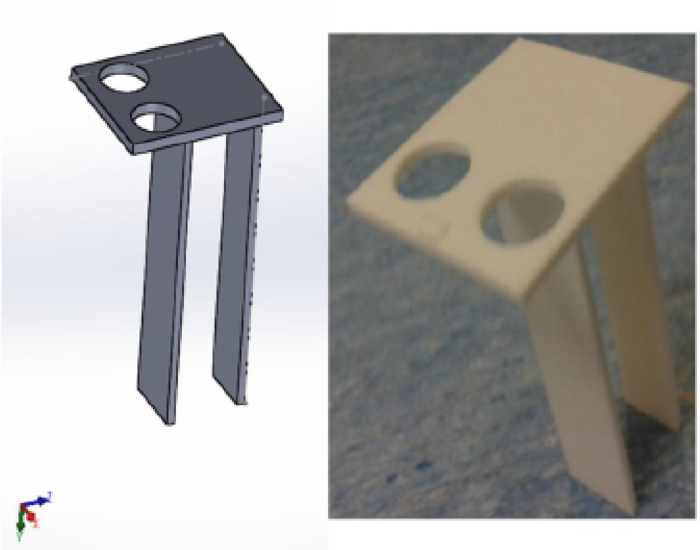

Figure 16: SolidWorks caption of the designed support piece for the US sensor (left), and the 3D printed PLA piece (right).

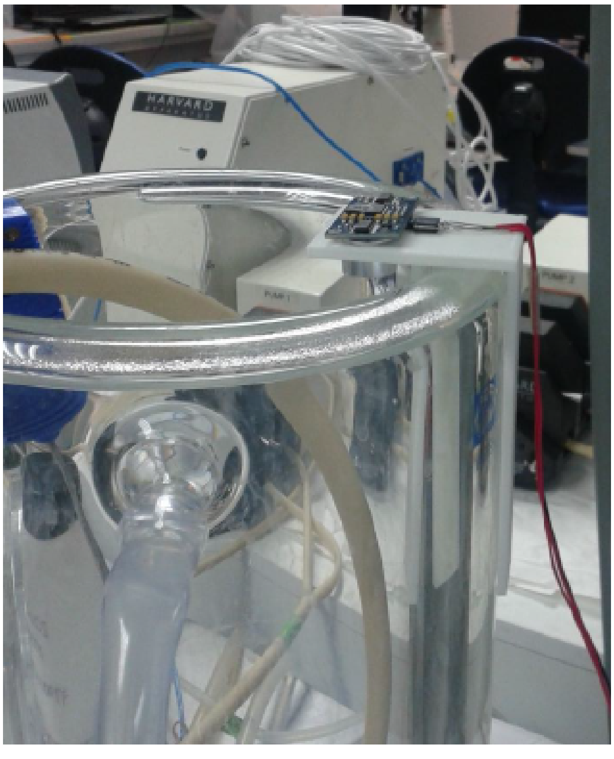

Figure 17: US sensor placed with the support piece on the vessel where the level is measured.

The level sensor proved to have a step response every centimeter, measuring exact centimeters properly, and changing the measurements approximately at half centimeters, showing an absolute error of measurement of 0.5cm at most. Above 18 cm from the half centimeter on, the measurement is the next higher value (i.e. 18.5cm retrieved a measurement of 19cm) and below this value the measurement is the lower value (17.5cm retrieved 17). For very close distances the sensor started malfunctioning, as shown below 4 cm. The trial did not reach a distance of 0 cm because the sensor is not waterproof and those close distances are out of the needed scope. The measurement contrast can be seen in Figure 18:

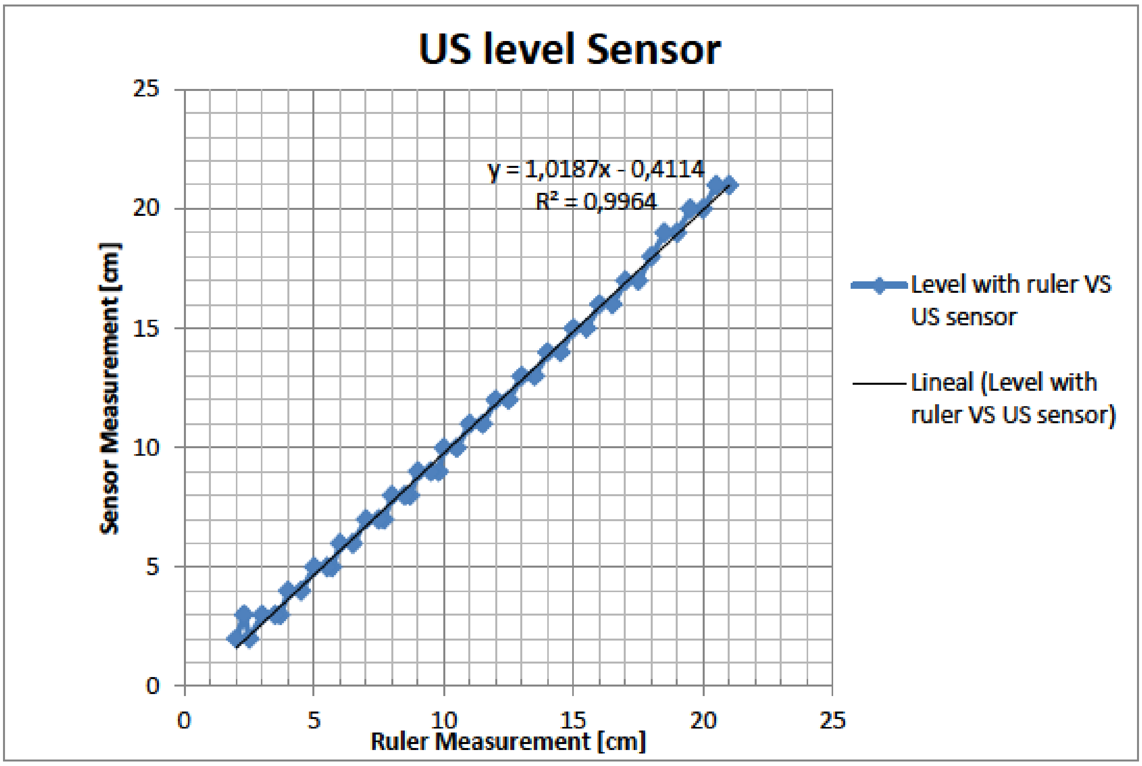

Figure 18: Measurements of ruler (gold standard) versus US Sensor used in this project for level measurement, on a vessel where water was added in 0.5cm steps

6.2. Threshold declaration for Oxygenation check

An intensity threshold was found that matched perfectly with the experiment light intensity, directing with a mirror some of the light beams directly to the small vessel were oxygenation was to be checked, with which the camera was able to capture bubbles with resolution enough to find correlation differences. For this specific setup this value was 0.5 in grayscale range (0-255).

With this intensity a correlation value threshold was found in 0.85 (being from 0 to 1 the range of correlation coefficients). Above this correlation value, the images were so similar, which meant that there were no bubbles flowing. With these thresholds the algorithm described in Figure 10 was able to detect effective oxygenation when the camera was placed pointing the small recipient as shown in Figure 19.

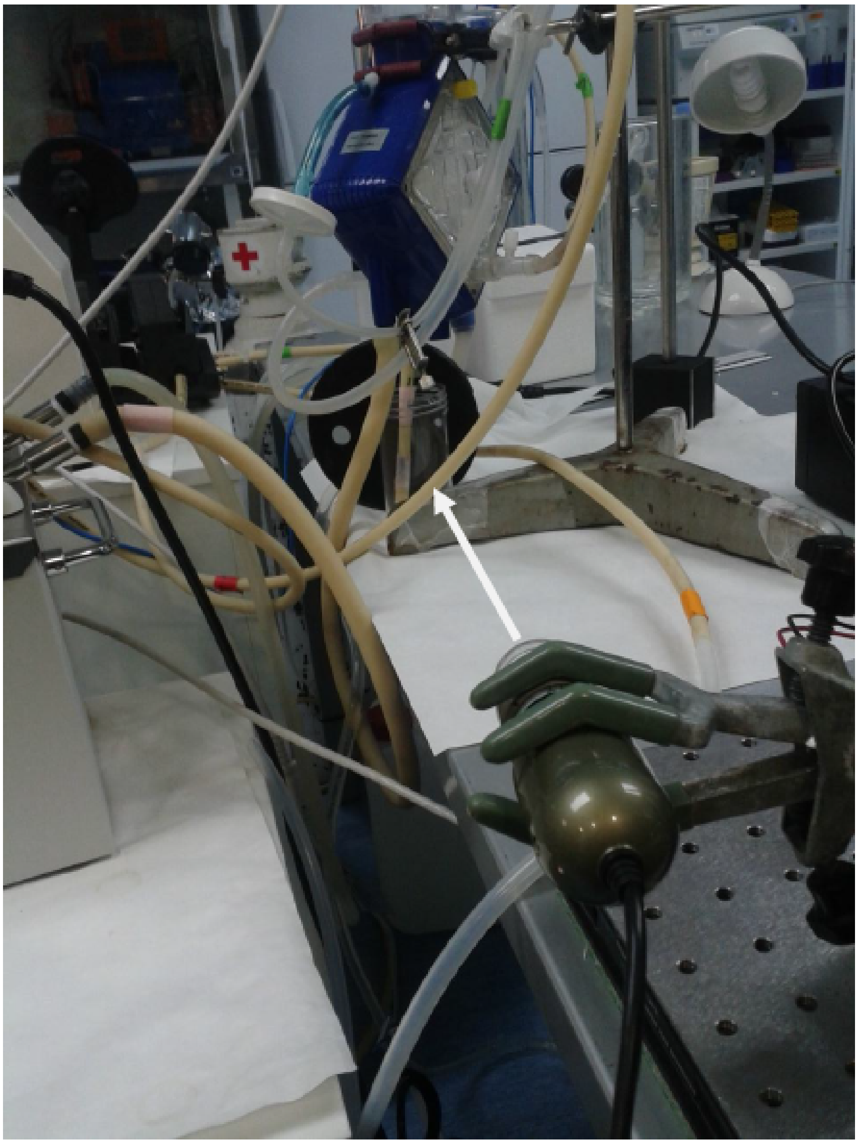

Figure 19: Camera placed facing the small recipient of media for image acquisition and oxygenation check algorithm proper functioning.

6.3. Assembly

All the connections were soldered properly and made contact as devised on the breadboard. The appearance of the assembled system can be seen in Figure 20.

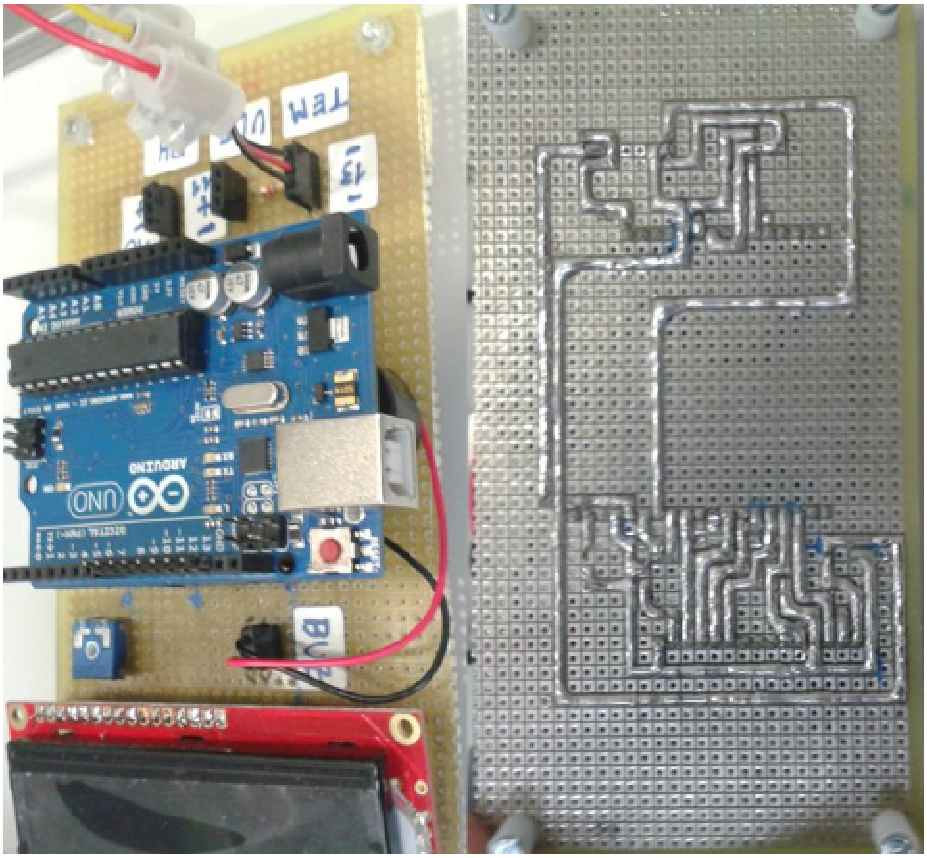

Figure 20: View of the system assembly. Top (left) and bottom (right).

The designed PCB using Fritzing software was correctly routed and all the paths were properly devised. Figure 21 is a screen capture of the software in which the PCB can be visualized.

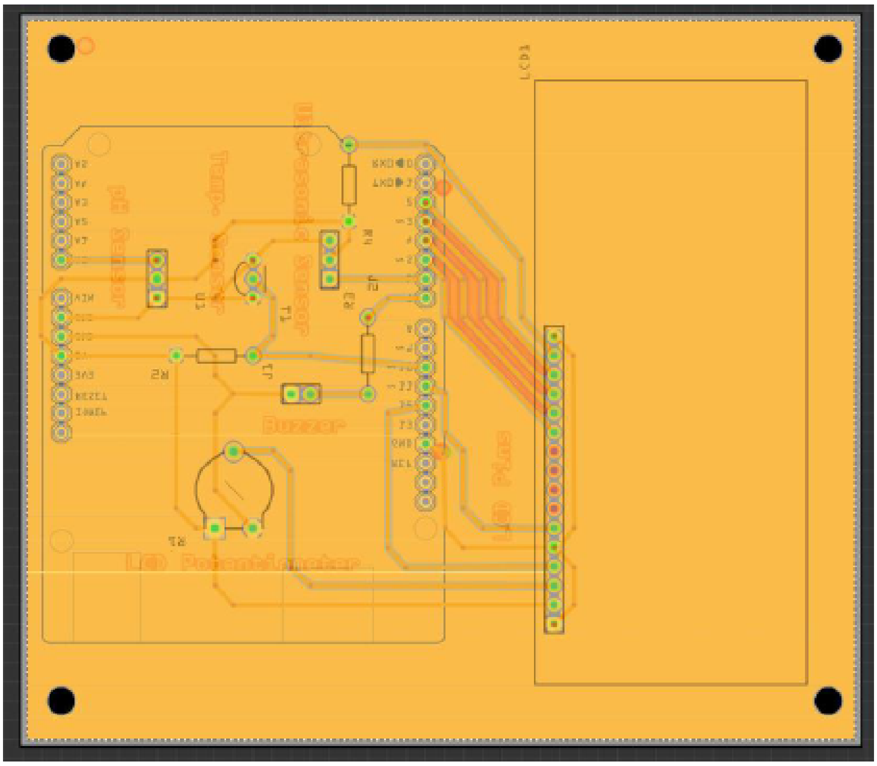

Figure 21: Screen capture of Fritzing software showing the designed PCB

6.4. Whole system compatibility and functioning

The system was kept for several hours working without crash. The user editable functions proved not to interfere with the measurements, as explained in detail below. Figure 22 shows a caption of the program running with some user editable options correctly working.

The reading was not carried out until the user decided so and ended when the Read button was switched off. In the same way, the recording could only start when the measurements had started, and the reading could stop when the recording had been stopped. The recording correctly stored a value for each parameter every five minutes, showing the date and time when it was recorded, in a text file and in a saved structure variable.

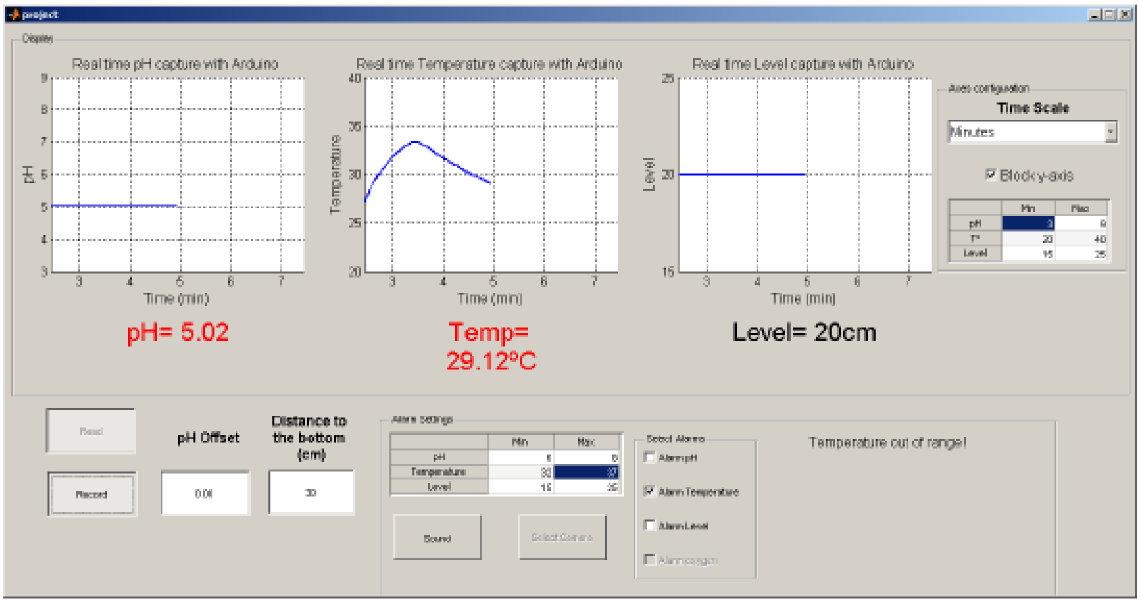

Figure 22: Screen capture of the system on execution showing the graph display setting changes, and alarm selection correct functioning.

Graphs could be edited to show different timescales and the range of values that the user wanted to see for each individual parameter, and this did not influence the measurements or the correct triggering of the alarm at any time, just how the data was displayed, as expected.

The system was able to operate with and without camera, the first supposing a longer period of time between measurements due to the time it took to get the images and process the data before asking for a new measurement. It correctly detected when the light was insufficient to measure. Also correctly detected whether the oxygen valve was open or closed when the illumination was high enough. However it failed sometimes in detecting it when the light intensity was way higher than the one devised for the experiment, as expected due to the increasing correlation threshold at high light intensities.

The alarm settings showed to work properly without interfering in the continuous measurements but complementing them. If any of the measured parameters was out of the user defined range in the editable table for the alarm limits, the text box showing the last measurement correctly switched font color to red. If the check box corresponding to each parameter was checked a sentence appeared in a text field telling which of the checked measurements was out of range. If the Sound button was toggled and any of the checked variables was out of range, apart from the indicative text, the alarm was triggered, or disabled if the problem was solved or the range was increased. Although the Sound button was toggled and a variable was out of range, if the check box corresponding to that variable was not checked, then neither the alarm was triggered nor the text did appear. However, the color of the textual display did change. **This proved the capability of enabling and disabling alarms at all with the Sound button and text with the check boxes at real time or just silencing a single parameter when the sound was on but the check box was not, all without the occurrence of any problem.** Oxygenation was only checked when its check box was checked, as programmed, to avoid time consumption when this measurement was not required by the user or a camera was not connected.

The message retrieved to Arduino to execute the measurement, correctly adapted to any input value and triggered/silenced the alarm without malfunction. The measurements shown in the LCD were exactly the same sent to MATLAB (Figure 23).

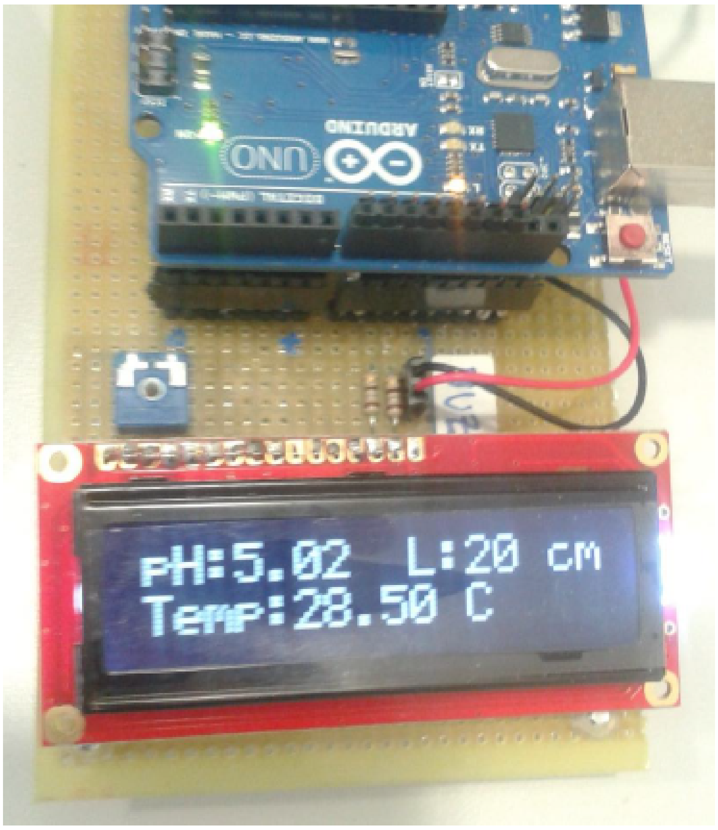

Figure 23: Measurements displayed on the LCD

In the case of the camera selection, the program detected all the available cameras that were already connected before opening MATLAB, but not those connected after.

7. Discussion

During this bachelor thesis a system to control physical parameters in a bioreactor has been developed. The equipment is able to measure pH, temperature and level of liquid medium on a vessel in real time, along with an oxygenation check on a reservoir. It has proven to work properly and has been installed in the bioreactor for which it was designed where it is being used on experiments.

This system contributes to the bioreactor with several enhancements. First it registers four parameters at the same time with a single user interface. It displays new measurements continuously and is able to keep a record of them for posterior analysis. This avoids the annotation of single measurements and the usage of different devices because automates the measurement process during the whole experiment, with an automatic complete display and record.

Despite being complete it poses the advantage to be mounted in a modular way, meaning that any sensor could be replaced without affecting the rest of the system. Changes may impose some recoding of the program, but just in the code concerning the replaced part. This advantage could be exploited if the system needed to be upgraded or if some unit in particular needed a replacement for more precise measuring.

This might be the case of the pH sensor. The objective of this unit is to check that the pH does not make a drastic change during the experiment, and for that purpose the provided response is more than enough, however, as mentioned in the results, the measurement has a rather high offset for the small scale that is comprised in the pH (from 0 to 14) and a slight non-linear behavior, so a further improvement could be done replacing this sensor by another one with a higher precision with the possible expense of a higher cost.

There are several alternatives for the pH measuring unit. First is the used potentiometric sensor. The potentiometric method, as briefly explained in the Materials and Methods section, uses a glass electrode in contact with the solution and a reference electrode. The glass bulb present on the tip of the electrode is permeable to the protons, and a change in pH of the solution will change the potential on the measurement electrode, producing a voltage difference proportional to the proton concentration [11]. Other alternatives are ISFETs (Ion Selective Field Effect Transistors) that change the allowed current through the transistor in function of the concentration of the selected ions and optical measurements of the color intensity of a pH sensitive dye [12]. Any of them is viable, however the optic measurement consumes dye every experiment and ISFETs have prices above 400€.

There exist also alternatives for the temperature sensor like the method of RTD (resistance temperature detector) in which the electrical resistance of a platinum wire, which is proportional to temperature [12]. In this case the obtained measurements are more precise and linear so a refinement of the precision could be done using the obtained linear fit instead of replacing the whole unit.

The level sensor is precise enough and would not make sense to replace it for a system with similar use, however it has a limitation: it is not waterproof. The assembly has been designed for a vessel with an opening that is neither the inlet nor the outlet and thus the fluid interfaces with open air, this limits its usage to this specific kind of bioreactors and should be replaced for those where the sensor must be in close contact with the liquid. In any case it fulfills the task of assessing wrong connections or leakage in the tubing and in a narrow vessel would show faster changes should some leakage happen and thus it will be more effective.

Using the camera to check oxygenation is an effective tool, and for the application it is given is more than enough. The thresholds should be calculated experimentally for each setup where this technique is going to be used, but if the illumination is enough for the camera to capture the bubbles it fulfills its task perfectly. The main drawback is that the measurement is qualitative and real percentage of oxygen cannot be calculated by these means. The sensors that measure the partial pressure of oxygen in solution could be a suitable replacement but they cost more than 350€, which is cheaper than the camera it has been used but very expensive in comparison to a regular camera. The camera that has been used was a microscope that was not being used for any task, but could be replaced by other cheaper camera. In blood based media the camera could be replaced by a pulse oximetry system.

MATLAB program is written in such a way that it could be edited to receive more parameters to display and control, as long as Arduino has enough pins to connect them. Arduino itself could be replaced by another microcontroller in the case that a higher sampling frequency was needed, but the measured parameters are not time dependent signals so Arduino suffices for this task, and perfectly connects to MATLAB to retrieve data.

Further developments could be the addition of more sensors, to the system, to have a more complete idea of what is going on while the medium is flowing. On the other hand, rather than having only alarms, a real control system with actuators could be implemented, to avoid abrupt changes that would imply problems in the course of the ongoing experiment or direct failure in properly nourishing the ex-vivo beating heart.

Another implementation could be a wireless network using ZigBee to distribute the sensors in a less restrictive way or to connect Arduino and the computer with a longer separation, allowing the two displays to be far apart without the use of a long wire, which may suppose an obstacle to the researchers.

8. Conclusions

The devised objectives were successfully met in the end of the project.

A unit of control of the pH of the medium was designed and developed, the real time measurements were fast and gave the possibility to keep an updated record of pH evolution over time.

A unit of control of the temperature of the medium was designed and developed too, giving a real time record of temperature.

A unit of control of the level of medium in the main vessel was also designed and developed successfully; retrieving real time measurements of the level with a good resolution for the use it was desired for.

The unit of control of the appropriate oxygenation degree was designed and developed through a specifically designed algorithm with Boolean result operating in an effective way.

A system was assembled, that incorporated the four aforementioned units and was capable to coordinate them and register and store these four physiological parameters controlled by the units.

A graphic user interface was designed using MATLAB that gave the user a real time display and the possibility to program alarms and control the output of the system.

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Annex I. Project User Manual

Needed Software:

- MATLAB 7.12.0 or higher.
- Arduino 1.0.5-r2

Archives:

- Arduino.ino charged in Arduino UNO
- project.m, project.fig, cameraSelect.m and cameraSelect.fig (in the same folder).

Sensors:

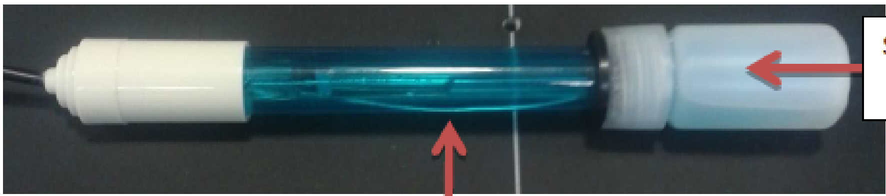
- **pH sensor (dfrobot sen0161)**

  Precautions during usage and storage:

  - The storage solution must be 3N KCl (potassium chloride 3 normal) and be about a pH=4.
  - Whenever you want to measure it is important to rinse the electrode in distilled water before sensing anything in order to remove any trace of the storage solution from it that could disturb the measurement. It is equally important to rinse it again in distilled water after any measurement and specially before taking it back to the storage solution to avoid changing its pH.
  - Calibration: it's composed of two steps, first introduce the electrode in a pH=7 buffer and observe the difference between the measured value and the real one (7!). If there is any difference you must edit the text field showed in the next section and put this value. Next (after cleaning the electrode!) you submerge the electrode in a pH 4 buffer and adjust the gain potentiometer of the sensor until the measurement is equal to the pH of the buffer:

Gain potentiometer of the pH sensor

LED signaling proper connection. If not lit, the connection is not making contact.



Storage solution

Electrode

(Introduce in the solution you want to measure after removing the small tube/cap containing the storage solution and after measuring put it in the storage solution tube again)

- **Temperature sensor (DS18B20 waterproof):**

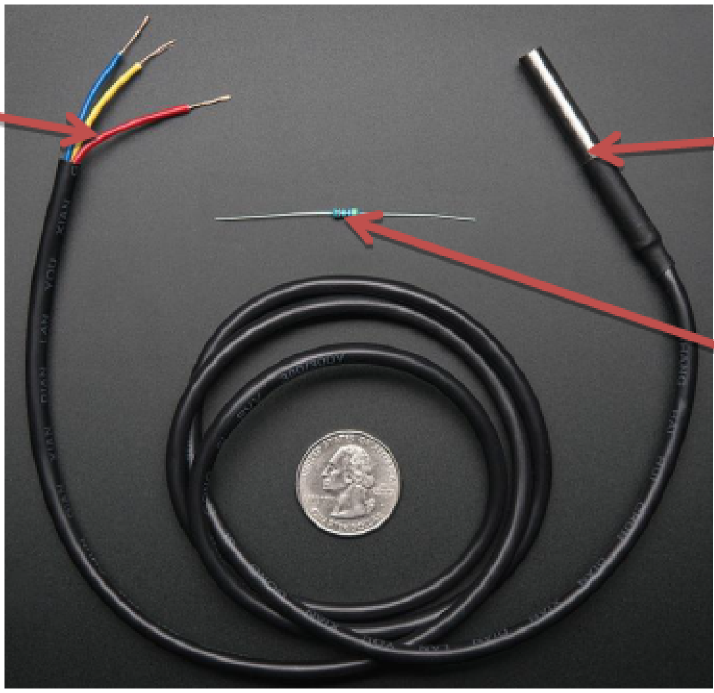
Connection cables:

Blue-GND

Yellow-digital I/O

Red-5V OR GND

(In our case the red cable is going to GND and works in parasite power mode)



Temperature sensor tip

Pull-up resistor

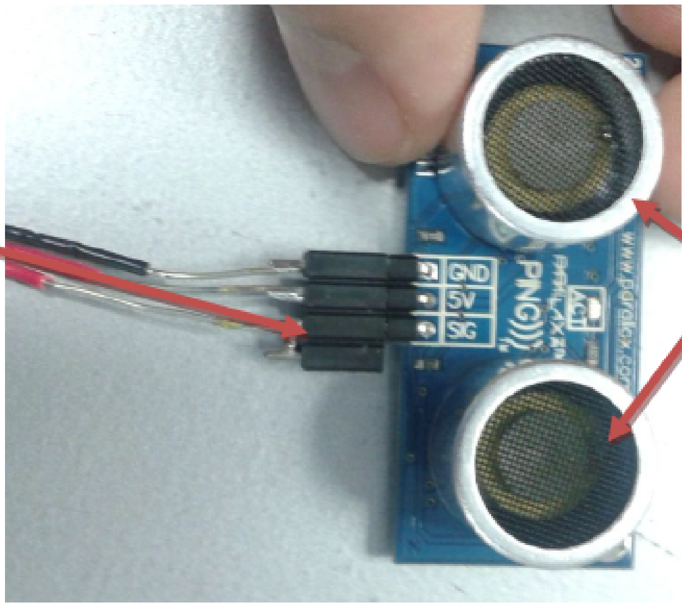
(It is connected between 5V and the data (yellow) cable, necessary for the sensor to work)

Precautions:

- To measure put the tip in contact with the liquid or material you want to know the temperature of.
- Clean and dry it with some cloth or paper after using it to measure the temperature of any solution or material that may have left the sensor dirty.

- **Distance Ultrasound Sensor (PING SEN-0012):**

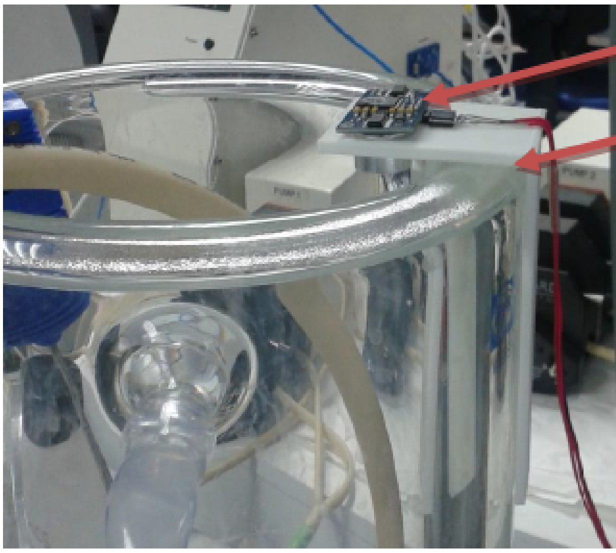
This is the distribution of wires for a soldered connector. 5V and GND are needed for the power supply, and SIG is used to transmit and receive the measurement.



Ultrasound transducers, in order to measure distance need to face in the direction of measurement.

Precautions:

- This sensor is able to measure with accuracy of centimeters, but for distances closer to 5 cm is not able to distinguish properly and retrieves 0 cm. Further distances are recommended to obtain accurate measurements and to protect the sensor, which is not waterproof.
- In order to measure the level of liquid inside the vessel, the measurement is subtracted to the distance from the sensor to the bottom of the vessel (height). This value has to be introduced by the user in a text field provided in the user interface.



Correct placing of the sensor on the vessel to perform measurements.

Physical Connections:

1. pH Sensor

- Red Wire is connected to 5V, + sign in the breadboard.
- Black Wire is connected to GND, - sign in the breadboard.
- Blue Wire is connected to A0 analog input, A0 sign in the breadboard.

2. Temperature Sensor

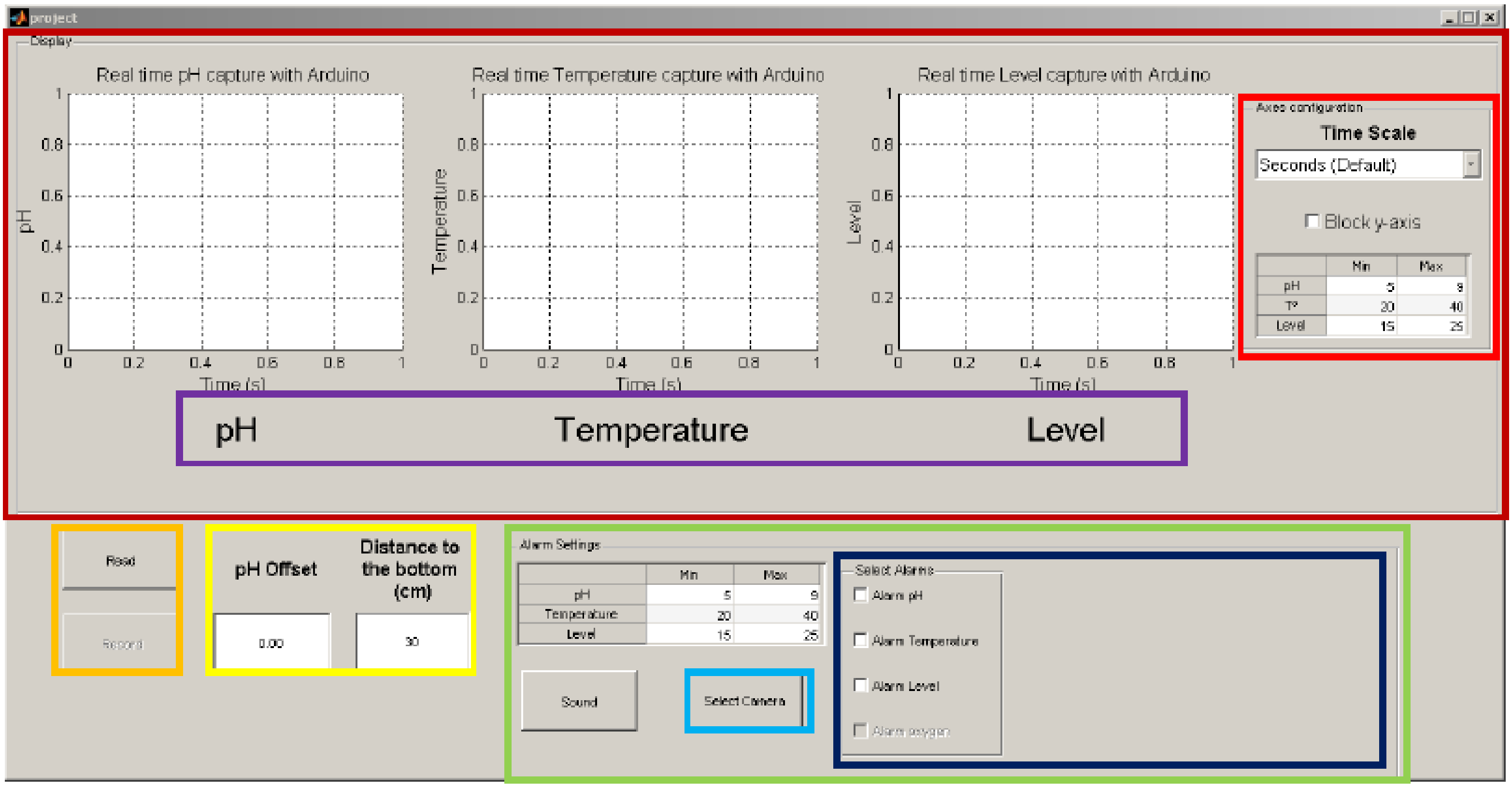
- Red Wire goes to 13 digital I/O, 13 sign in the breadboard.
- Both Black Wires go to GND, - signs in the breadboard.

3. US Sensor

- Black Wire goes to GND, - sign in the breadboard.
- One of the Red Wires goes to 11 digital I/O and the other to 5V. The three wires are labeled in order to avoid confusions because of color similarities on the tips. The wire connected to SIG connector in the sensor must go to 11 in the breadboard and the one connected to 5V to + in the breadboard.

Running the program

1. Open MATLAB and run project.m
2. This is the window that appears:



In order to understand better what we are seeing the different areas of this window are going to be explained referring to them by the color of the rectangle that encloses them.

- **Dark Red** is the display panel, in here is where the measurements are going to be presented in a real time graphs, where the user is able to see the time evolution of each parameter, and in the text fields below (enclosed in the **Purple** rectangle) the last value measured for each parameter. These values are normally presented in black font, but if they are out of the **alarm range** they will change to red, this will be better explained later.
- In **Bright Red** are the settings for the **graph display**. You can select from the **Time Scale** menu the units for the time axis of all the graphs. If the box **Block y-axis** is not checked, the last measured

value is displayed in the middle value of the graph, and the upper and lower limits of the measurements are 2 units above and below the measured value. If it is checked, the range displayed in each graph can be defined by the user in the **editable table**. Each row stands for each graph and the columns define the lower and upper limits of the range. (This changes that the user make must be coherent, in the **Min** column cannot be introduced a value higher than the one in the **Max** column, and vice versa.)

- In Green is the Alarm Settings panel. Here the user can choose the range of valid values for the measured parameters in the same way that the display range could be set, using the **table** in this panel. If the measured value is out of these ranges, the text display inside Purple will switch their font color to red. The **Sound button** activates or deactivates the sound alarm when it is toggled/not toggled. Inside **Dark Blue** is a text field and a checkbox for each parameter. If the checkboxes are checked and the measured value is out of range it will display a warning in the text field, and if the **Sound** is on it will trigger the alarm. This way the user can decide which parameters trigger the alarm and which do not.
- In Bright Blue is the **Select Camera button**, which runs the cameraSelect.m program. This program will be explained in a later section, but basically, allows the user to select from all the connected cameras to the computer to check the oxygenation qualitatively. After the camera selection, the **Check oxygenation** checkbox is enabled. A camera can only be selected when the program is not measuring.
- In Orange are the **Read** and **Record** buttons. These are the principal buttons for the program to run. When **Read** is toggled, Arduino-MATLAB communication start and measured values are displayed in real time. Also **Record** is enabled. If **Record** is toggled, every 5 minutes a record of the last measurement is added to a **text file** (History.txt) and a **structure variable is saved** with all the recordings

for each session. Reading cannot be stopped while recording; first the recording must be finished.

- In Yellow are two editable values, which are offsets for the pH measurement and the US proximity measurement respectively. In a later calibration section, what this values stand for is explained.

3. When all the sensors are placed properly (following section), the reading can start. Press the **Read Button**, wait 5 seconds for establishing the serial port connection and values will appear on the screen in the display (**Dark Red**) panel. If you want to keep a record of the values then press **Record Button**. To activate alarms for any parameter, press the **Sound Button** and check the **CheckBox** of the corresponding parameter. Edit the values on the **table** to define the ranges (Green enclosed panel). To change the way the graph is displayed, edit the values in the **Bright Red** panel (**Time scale**, **block y-axis** and the **table**). All these settings will not affect the recording of data. If you do not want any sound alarm, un-toggle the **Sound Button**, and if you do not want sound for a certain parameter check out the corresponding **CheckBox**.

4. To close the program un-toggle the **Record Button** if the recording is running, un-toggle the **Read Button** (finish recording and reading), and close the window.

   Additional Note: Should the program be closed while recording (due to computer crash or blackout for example), another variable called "Recordinghistory_temp.mat" will appear stored on the folder of the MATLAB path, containing all the recorded measurements up to the moment of forced closure.

cameraSelect (User interface and function)

1. This program is triggered when the **Select Camera Button** is pushed. It automatically detects all the cameras connected to the computer and allows selection of camera and resolution.

First select the **adaptor** and then the **available devices** appear in another menu. When the camera is selected, another menu with the **possible resolutions** appears and pressing the **Select Camera Button** in this window a preview for the camera will appear. Any camera and resolution can be tried this way until the correct one is chosen. Once this happens press **Close Button** to retrieve the camera identifier and settings to the main program. In the case of this system the adaptor will be **winvideo** and the camera **DINO Lite AD4113TL**. The resolution is up to the user but good results are obtained with the second best one.